The MacroScreen Platform

Chapter 8

Valorization

Valorization, defined as a "process that aims at enhancing societal impact"[1], is an important aspect of (academic) research. It is crucial for scientists to make the knowledge acquired during a PhD available and accessible to society both by transferring the results to potential stakeholders (e.g. pharmaceutical companies or other research centers) and if opportune, by translating them into possible news products that society could benefit from. In this chapter, we will present how the work described in this thesis has and will be transferred to society and how it, in the future, could help to reduce the socio-economic burden of cardiovascular disease.

Cardiovascular disease

Cardiovascular disease (CVD), mainly driven by atherosclerosis, is still the leading cause of death in the world[2]. In 2016, almost 18 million people died from CVDs globally, mostly attributable to coronary artery disease and stroke[3]. CVD has become an obvious economic burden, as the European Heart Network has estimated the total CVD-related cost to be €210 billion per year[2]. The main costs generated from CVD are related to healthcare of CVD patients (healthcare, medication, hospitalization), but also indirect costs, such as patient work productivity loss and intangible costs, e.g. costs of pain and suffering, are substantial. This highlights the need to rapidly discover new therapies or strategies to cure and study CVD. CVD research centers on two major topics: (1) investigations on mechanistic pathways involved in the pathophysiology of CVD; (2) translational studies aimed at CVD diagnosis, prevention and intervention, often based on findings from the basic science studies. In this thesis, we aimed to cover both areas of research, with **chapter 3 and 5** focusing on prediction and prevention of CVD, and **chapter 2 to 4** mainly focusing on understanding functional pathways involved in CVD inflammation.

As described above, valorization can be accomplished via two main tracks: "societal transfer of results" and "economical transfer". We have achieved the former by presenting the main results described in this thesis at (inter)national conferences. In addition, making scientific knowledge accessible to everyone is another key aspect of societal transfer. During my PhD, I was selected to give a Ted-talk at the PhD-student course organized by the Dutch Heart Foundation in 2017, improving my communication skills to present scientific research to a non-scientific audience. We further intend at disseminating the acquired knowledge to cardiovascular

patients by giving lectures via the Harteraad (https://www.harteraad.nl/). Moreover, we aim at publishing the main results of this thesis in peer-reviewed, preferably open access, scientific journals; in fact **chapter 4 has** already been published in an open-access journal, and **chapter 5** is under review at European Heart Journal. Datasets will be deposited in repositories that are publicly available, such as GEO. The published results could then be used as starting point or reference for the scientific community in future research. As to the second track, the translation of results into potential diagnostic or therapeutic strategies to detect and treat CVD, we aim at conducting an active Intellectual Property IP protection strategy to allow successful knowledge exploitation of the projects described in this thesis. Together with carefully selected external partners, such as biotech companies, we will develop a cost-effective utilization strategy of, for example, our MacroScreen platform or our predictive lipid-signature. The below paragraphs will expand the different valorization opportunities of the main results of this thesis.

Bringing the predictive lipid signature to the market

In **chapter 5**, we have profiled circulating lipids in two independent obese patient cohorts and built a prediction model of future CVD. Obesity is a well-known risk factor for CVD; it accounts for approximately 30% of the cases[4]. This is explained by the "bi-angular relationship" between CVD and obesity; obesity is both directly and indirectly linked to CVD due to the abnormal blood lipid profile and to its relation to other important risk factors of CVD, such as hypertension and diabetes, respectively. However, current risk factors, such as cholesterol levels, blood pressure or glucose levels, lack predictive power for the development of CVD in the obese population to be useful for individual risk assessment. The lipid-signature described in **chapter 5,** has unprecedented predictive power for future CVD and could be a valuable tool for early detection of high-risk obese patients, motivating them to lifestyle changes, instead of costly life-long medication, such as statins, or pricey surgical operations, thereby reducing the economic burden of CVD.

Current risk prediction is performed by calculating risk scores, based on many clinical parameters. Ideally, our multi-lipid CVD risk signature could be translated into a fast and accurate *in vitro* diagnostic (IVD) test for plasma samples of obese patients, which then would be used in the clinics to further help in individual CVD prediction. However, translating these findings to an accurate IVD test requires further study to prove the diagnostic value of our prediction model. First, future

studies will have to reproduce and validate these findings in even larger cohorts, as patient numbers still were rather limited for both our discovery (n=78) and validation cohort (n=200). Second, current lipid profiling is performed using mass spectrometry. This technique requires specialized infrastructure and is expensive and time-consuming, making it not feasible for point-of-care testing. Therefore, further studies aim at developing an IVD assay, which could for example rely on aptamer technology, to detect our lipid signature. Aptamers arrays are spotted with single strand DNA or RNA (ssDNA/ssRNA), that have a unique tertiary structure able to specifically bind to a single lipid[5] and have already showed their potential for biomarker discovery[6]. Together with specialized companies, such as Novaptech or Eurogentec, which have experience with production of aptamer-based arrays for diagnostic purposes, we will develop and validate a diagnostic product at different location sites and on a high number of biological samples. This easy-to-use diagnostic tool could directly be used by general practitioners and in the hospital, and lead to early identification of high-risk CVD patients, early individualized intervention and CVD prevention. Next to this, this diagnostic tool will help monitor medical treatment efficacy and will be used as an outcome measurement for clinical trials and intervention studies.

Potential therapeutic solutions

Current medication for CVD involves lipid lowering therapy, including statins[7], and antihypertensive treatment. Despite their effectiveness with a 20% reduction in CVD risk, lipid lowering therapies are unable to prevent future cardiovascular events in a significant number of patients[8]. More recently, intervention in IL-1β signaling with a monoclonal antibody on top of statin treatment, in subjects with low grade inflammation led to a supplementary reduction of 14% in CVD incidence[9]. However, this was accompanied by increased infection numbers and, importantly, no difference in overall mortality was observed after IL-1β antibody treatment. This emphasizes the need to discover new therapeutic targets to decrease CVD risk and develop new diagnosis tools to enable detection of high-risk profile at a reversible stage of the disease.

In **chapter 5**, we discovered an 18-lipid signature predicting the incidence of CV events in the obese population. Using our MacroScreen platform, we could show that some of our lipid predictors were affecting key functions of macrophages, the main immune cell type implicated in atherosclerosis, hinting at a possible causal

relationship. Moreover, these lipids were enriched in unstable, as compared to stable human atherosclerotic plaques. Future research aims at studying the implication of these lipids in disease development and progression, by interacting with their synthesis *in vivo* for example. This could potentially lead to the discovery of new key players in atherosclerosis related CVD and, subsequently, open new avenues for therapeutic solutions. As statins fail at reducing the risk in a majority of patients, despite their profound effect on cholesterol and, indirectly, on other lipids levels[10], novel drugs should be generated. To target specific lipids, one could think of generating molecules interacting with their synthesis pathway by inhibiting a key enzyme, for example.

Another potential target for future intervention was unveiled in **Chapter 4,** where we investigated the impact of a deficiency of Mcl-1, an anti-apoptotic protein, on atherosclerosis. We were able to convincingly show that Mcl-1 is essential for survival of macrophages, as well as neutrophils. Additionally, Mcl-1 was implicated in macrophage lipid uptake and subsequent macrophage fusion capacity, resulting in the formation of multinucleated giant cells (MGCs). Evidence for the presence of MGCs in human atherosclerotic plaques is scarce; and the description of a clear role in the pathogenesis of disease is lacking. One study described MGCs to express high level of cathepsins, enabling the rupture of the elastic lamina and facilitating the migration of smooth muscle cells during atherosclerosis[11]. Clearly, more research is needed to understand the implication of MGCs in atherosclerosis and their exact role in disease development and progression. Second, small molecules targeting Mcl-1 already exist, as Mcl-1 is an interesting target to treat cancer, due its high expression in tumor cells and role in cell survival[12,13]. However, as we showed, targeting Mcl-1 in an animal model of atherosclerosis led to severe neutropenia, and had no effect on atherosclerotic lesion size. Therefore, targeting Mcl-1 will most probably not lead to successful therapeutic strategies; yet, this could be used as a model of neutropenia or giant-cell enriched atherosclerosis to further investigate MGCs implication in atherosclerosis.

Novel human centered validation strategies in CVD research

Funding for cardiovascular disease research is sizable[14]. Between 2010 and 2012, funding for CVD research (both academic and in private company setting) amounted around €876 million in Europe[14]. CVD research is nowadays mainly performed on animal models, which are expensive, and time consuming and do

not, in every aspect, represent human disease. Therefore, building a humanized test platform to capture cardiovascular inflammation, such as atherosclerosis and AMI, in *vitro* (**chapter 2 to 5**) could help reducing the costs of CVD fundamental research and drug development. First, **chapter 2** showed that macrophages are functionally reprogrammed after exposure to various stimuli, such as several cytokines or fatty acids. This led to the identification of a macrophage activation functional map, which could serve as reference for further studies. Second, **chapter 3** investigated the functional responses of macrophages after exposure to AMI patient serum. We observed that macrophages functionally respond to AMI systemic environment and these functionalities were also linked to poor prognosis, four months after the infarct, showing the potential utility of this platform as a fast and cheap drug screening platform. One could think of assessing the effect of compound libraries on key functions of macrophages related to AMI systemic environment, and subsequently select the most interesting target for further analysis. Importantly, our platform will never serve a diagnostic purpose, as it is too time-consuming and labor intensive for clinical use.

Altogether, this demonstrates that our platform has great potential to quickly study the implication of macrophages in CVD inflammation and to screen for modulators of critical macrophage functions for drug development purposes. The platform is ready-to-use, however future research aims at improving its physiological complexity, as it is currently based on two-dimensional monolayers of cells and this lacks the cell-cell interaction and 3-dimension aspects of the human body.

Conclusion

In conclusion, the work presented in this thesis aimed to tackle the socio-economic burden of CVD at several levels. We have developed a new tool, able to characterize macrophage functions in the context of CVD inflammation. This tool could, in the future, help reduce the use of animal models and improve the translational problem linked to animal studies. Furthermore, we have identified a predictive lipid signature for the development of obesity related CVD, which shows promise for stratifying obese individuals with increase CVD risk and may also have therapeutic potential. Lastly, we have investigated the impact of Mcl-1 in atherosclerosis, and its implication in giant cell formation opening new avenues to manipulate MGC formation in atherosclerosis, and potentially other diseases where MGCs play a role

References

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